

Romer Labs
AgraStrip Total Aflatoxin Quantitative Test WATEX

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GENERAL INFORMATION

The AgraStrip Total Aflatoxin Quantitative Test WATEX is a one-step lateral flow immunochromatographic assay for the quantitative screening of total aflatoxin in corn samples. The test is based on a competition immunoassay format. Antibody-colloidal gold complex (conjugate) lyophilized in a microwell is mixed with sample extract. An aflatoxin strip is placed into the microwell. The mixed content is then wicked onto a membrane of the total aflatoxin strip, which contains a test zone and a control zone. The test zone captures free antibody-particle complex (conjugate), allowing color particles to concentrate and form a visible line. The color intensity of the line is inversely proportional to the concentration of total aflatoxins in the sample. The line is always visible in the control zone regardless of the presence of total aflatoxins. The aflatoxin strips are measured using an AgraVision Reader and the results are determined.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	<i>Romer Labs, Inc. (636) 583-8600</i>
Test Kit Name:	AgraStrip Total Aflatoxin Quantitative Test WATEX
Product Number:	COKAS1600W
Effective Date of Instructions:	07/28/2015
Instructions Revision Number	5
Conformance Range:	5 – 100 ppb
No. of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	No
Approved Commodities:	Corn, corn flour, corn flaking grits, corn grits, distillers dried grains with solubles (DDGS), soybeans, and wheat.

Extraction method:	Shake vigorously 50-gram sample with 150 milliliters (mL) of water and one buffer packet by hand for 2 minutes at 200 – 250 oscillations per minute or shake with similar shaking action (as hand shaking) for 2 minutes using a mechanical shaker.
Test Format:	Lateral Flow Strip
Detection Method:	AgraVision Reader (Product Model No. EQASR1000)

PREPARATION OF TESTING MATERIALS

All reagents and kit components must be at room temperature 20-24°C (68-75°F) before use. The temperature of AgraStrip Incubator is set at 45°C.

Note: It is recommended to switch on the incubator in the morning and to keep it on throughout the whole day.

SAMPLE PREPERATION AND EXTRACTION PROCEDURES

Standard Extraction Procedure for corn, corn flour, corn flaking grits, corn grits, distillers dried grains with solubles, soybeans, and wheat:

- (1) Weigh out 50.0 ± 0.2 grams ground sample into one side of a filtering Whirl-Pak bag.
- (2) Place one packet of soluble buffer onto the ground sample in the Whirl-Pak bag.
- (3) Add 150 mL of distilled or deionized water and securely close Whirl-Pak bag.
- (4) Shake closed Whirl-Pak bag vigorously by hand for 2 minutes at 200 – 250 oscillations per minute or shake with similar shaking action (as hand shaking) for 2 minutes using a mechanical shaker.
- (5) Allow sample to settle for 2 minutes to get filtered extract (extract can only be used for next 5 minutes).

Dilution Procedure for corn, corn flour, corn flaking grits, corn grits, soybeans, and wheat:

- (a) Dilute the filtered extract 1:21 with dilution buffer. (i.e. use a blue pipette tip to add 1000 μ L of Dilution buffer to a dilution tube. Then, use a yellow or white pipette tip to add 50 μ L of extract into the dilution tube).
- (b) Mix well and the sample is ready for assay (this diluted sample can only be used for next one hour).

Dilution Procedure for Distiller's Dried Grains with Solubles:

- (a) Dilute the filtered extract 1:31 with dilution buffer. (i.e. use a blue pipette tip to add 1500 µL of Dilution buffer to a dilution tube. Then, use a yellow or white pipette tip to add 50 µL of extract into the dilution tube).
- (b) Mix well and the sample is ready for assay (this diluted sample can only be used for next one hour).

TEST PROCEDURES

a. Analysis Procedure

- (1) Place the cover of the heat block on the top of the heat block. Break off the appropriate number of conjugate coated microwells for the samples to be run. Remove sealing caps from conjugate microwells, and place the conjugate microwells inside the heat block. Ensure that the wells are fully seated in the heat block. **Only one sample should be run at one time.**
- (2) Add 100 µL of diluted sample extract to each conjugate microwell.
- (3) Mix the content in each microwell by pipetting it up and down 10 times.
- (4) Put one test strip into one microwell. Place the cover back into the heat block to cover the microwells and test strips.
- (5) Allow the test strip to develop color for 3 minutes. Lift the heat block cover and place it on the top of the heat block.
- (6) Wipe the end of the strip test onto an absorbent paper and insert the strip into the strip holder/tray for reading.

b. Reading the Results

- (1) Use the AgraVision Reader to read the strip and interpret result. **Note:** follow the instruction of AgraVision Reader to read the strips. Strips should not be read more than 1 minute after completion of the run.
- (2) Turn on the AgraVision reader using the power button located on the back.
- (3) Use the arrow keys on the keypad to highlight “TEST”. Select it using the checkmark key.
- (4) Use the arrow keys on the keypad to highlight “Mycotoxin”. Select it using the checkmark key.
- (5) The barcode scanner will turn on. Scan the barcode, found on the microwell tube included in the test kit.

- (6) Create a sample ID by using the alphanumeric keys on the keypad. Use the checkmark key to enter.
- (7) Since only one strip is being read; use the pound key to bypass to the next screen.
- (8) Enter the operator ID. Press the checkmark key to enter, and press it a second time to move to the next screen.
- (9) The reader is ready to read and will display “start measurement”. Insert the strips into the tray, and insert the tray into the reader. The strips should go in the tray with the white end facing into the reader. Press the checkmark key to read.
- (10) After completion of reading, press the checkmark key to save the result in the AgraVision Reader’s memory or the pound key to print the result.

Note: after the test, the used microwells can be removed with a tweezers provided with the kit.

c. Interpretation of Results:

- (1) A color line always appears in the upper section of the test strip to indicate that the test strip is working properly. This line is the Control Line (C). A line in the lower section of the test strip indicates the test result. This line is the Test Line (T).
- (2) **Invalid results:** If there is no control line in the control zone, the test is invalid and the sample should be re-tested by using a new test strip.
- (3) **Valid results:** 2 lines are visible. The intensity of the line in the test zone is dependent on aflatoxin concentration in the sample and must be measured with an AgraVision Reader.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@usda.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

- (1) Store test kits at 18-25°C (64-77°F) when not in use, and do not use beyond the expiration date. Do not freeze. Do not leave it in direct sunlight.
- (2) Test strips must be kept inside their original tubes.

- (3) Conjugate microwells must be kept inside their original tubes.

b. Precautions

- (1) All reagents must be at room temperature before the assay is running.
- (2) Adhere to the instructions of test procedures.
- (3) Do not re-use test strips and conjugate wells.
- (4) Consider all materials, containers and devices that are exposed to the sample to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
- (5) The components in this test kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

EQUIPMENT AND SUPPLIES

a. Materials Supplied with the Kit:

- (1) 1 tube containing 24 AgraStrip Aflatoxin test strips
- (2) 1 tube containing 24 AgraStrip Aflatoxin WATEX Conjugate wells with lyophilized antibody particle complex (conjugate)
- (3) 1 bag containing 24 AgraStrip WATEX Extraction Buffer Bags.
- (4) 1 bottle of 30 mL AgraStrip Aflatoxin WATEX Dilution Buffer
- (5) 1 bag of 48 yellow or white pipette tips, 1 bag of 24 micro centrifuge tubes (dilution tubes)
- (6) 24 Filter Whirl-Pak bags, 1 pair of blue tweezers

b. Materials Required but not Provided with Kit

- (1) Romer Series II Mill or equivalent, EQOLE1010: Balance, 400 grams
- (2) EQOLE1050: 250 mL Graduated cylinder, Distilled or deionized water

c. Assay Procedure

- (1) Single channel pipette capable of pipetting up to 100 μ L.
- (2) Single channel pipette capable of pipetting up to 1000 μ L.
- (3) EQOLE1300: Timer

- (4) EQASR1003:AgraVision Reader without printer or EQASR1000: AgraVision Reader with printer
- (5) EQOEV2060: AgraStrip Incubator with timer or EQASR1005: AgraStrip heat block with cover.

REVISION HISTORY

Revision 5 (07/28/2015)

- Wheat and soybean were approved for this test kit and the test procedures of these commodities are added in this version.

Revision 4 (05/14/2015)

- Described the shaking procedure in more details as “ Shake closed Whirl-Pak bag vigorously by hand for 2 minutes at 200 – 250 oscillations per minute or shake with similar shaking action (as hand shaking) for 2 minutes using a mechanical shaker.”

Revision 3 (03/30/2015)

- Corn flour, corn flaking grits, corn grits, and distillers dried grains with solubles were approved for this test kit, and the test procedures of these commodities are added in this version.

Revision 2 (01/07/2015)

- Correct Acronym of Policies, Procedures, and Market Analysis Branch (PPMAB) has been used.
- Phone number of Patrick McCluskey (816-659-8403) has been corrected.

Revision 1 (12/03/2014)

- Removed Carl Jackson as contact.
- Added Dr. Ajit Ghosh as contact for the Technology and Science Division.